

Overwintering with a low level of cold-hardiness in the temperate millipede *Polydesmus angustus*

J.-F. David (*), M.-L. Célérier (**) and G. Vannier (***)

(*) Centre d'écologie fonctionnelle et évolutive, CNRS, BP 5051, 34033 Montpellier, France.

(**) Institut d'Écologie (URA 258 CNRS), Université de Paris 6, 75252 Paris Cedex 05, France.

(***) Laboratoire d'Écologie générale (URA 1183 CNRS),
Muséum national d'histoire naturelle, 91800 Brunoy, France.

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Abstract

Tolerance of freezing and ability to supercool were investigated in all stadia of the flat-backed millipede *Polydesmus angustus*. When cooled at a rate of $1^{\circ}\text{C min}^{-1}$, individuals survived sub-zero temperatures above the supercooling point (SCP), but did not tolerate freezing. SCPs were comparatively high, with a mean value of -5°C in winter and individual SCPs as high as -2.2°C in the largest specimens. Millipedes starved for three weeks in the laboratory did not depress their SCP. It is thus concluded that *P. angustus* has virtually no physiological protection against freezing.

In cultures under two thermal regimes – with or without moderate frost – nearly all millipedes survived throughout the winter at temperatures just above 0°C but 100% mortality occurred when temperature decreased to c. -6°C . Although the possibility of death from chilling cannot be excluded, the results are consistent with the supercooling profile of the species and confirm its low level of cold-hardiness.

In the field, *P. angustus* is commonly found in rotting logs, which have been shown to provide considerable thermal buffering in winter. The benefits (possibility of feeding throughout winter) and limits (in terms of survival and geographical range) of the overwintering strategy of *P. angustus* are discussed. The assumption that physiological resistance to freezing should decline in species which avoid frost by behavioural means is examined. The evidence is conflicting in insects, but clearer patterns may emerge from studies on other classes of arthropods.

Keywords: Freezing, supercooling, winter survival, microclimate, geographical distribution.

Résumé

La tolérance à la congélation et la capacité de surfusion ont été étudiées chez le Diplopode *Polydesmus angustus* à tous les stades du développement. Lors de refroidissements à la vitesse de $1^{\circ}\text{C min}^{-1}$, tous les individus survivent aux températures négatives supérieures au point d'abaissement cryoscopique (SCP) mais meurent dès que ce point est dépassé. Le SCP est relativement élevé, avec une moyenne de -5°C en hiver et des valeurs individuelles atteignant -2.2°C chez les plus grands individus. De plus, des Diplopodes privés de nourriture pendant 3 semaines au laboratoire n'abaissent pas leur SCP. On en conclut que *P. angustus* est pratiquement dénué de protection physiologique contre le gel.

La comparaison de deux séries d'élevages, les individus étant exposés ou non à des gelées modérées, montre que la mortalité hivernale des Diplopodes est très faible lorsque la température reste légèrement positive, mais que la mortalité est de 100 % lorsque la température descend aux environs de -6°C . Ces résultats sont conformes au profil cryoscopique de l'espèce et confirment sa faible résistance au gel.

Dans la nature, *P. angustus* hiverne généralement dans le bois pourri, où la température reste très supérieure à celle de l'air ambiant en cas de gélée temporaire. Les avantages et les inconvénients d'une protection purement comportementale contre le gel sont examinés. L'hypothèse d'une corrélation étroite entre le niveau de protection physiologique des espèces et le niveau des risques encourus dans leur microhabitat est discutée.

INTRODUCTION

Many studies have dealt with the physiological aspects of the resistance to freezing in terrestrial arthropods, especially insects. Two distinct processes occur: (i) freezing-tolerance, which makes it possible to survive ice formation inside the body but is restricted to a minority of species; (ii) avoidance of freezing by supercooling, which maintains the body fluids in the liquid state at low sub-zero temperatures and is much more widespread (SALT, 1961; ZACHARIASSEN, 1985; CANNON & BLOCK, 1988; LEE, 1991). Some insect species from extreme climates also combine the two (RING, 1982).

However, many arthropods from temperate regions escape freezing mainly by behavioural means (DANKS, 1978; BALE, 1987). This is obvious in certain soil dwellers such as julid millipedes, which burrow at depths where it never freezes (HAACKER, 1968). In such conditions, DANKS (1978) hypothesized that physiological adjustments, being metabolically costly, should not persist if not needed for survival. Although costs related to resistance to freezing have been demonstrated – e.g. production of cryoprotectants and antifreezes, cessation of feeding and, at least for freezing-tolerant animals, costs of being non-mobile at high sub-zero temperatures (LEATHER *et al.*, 1993) – the empirical evidence in support of DANKS' hypothesis remains limited. Among insects overwintering in the soil, some species show both an absence of freezing-tolerance and a high supercooling point (HOSHIKAWA *et al.*, 1988), but they are the exception rather than the rule (SØMME, 1982). Many temperate insects can supercool much more than they need to.

From this viewpoint we have begun to study millipedes (Diplopoda) overwintering in protected microhabitats in western Europe. The first species investigated, *Polyzoniium germanicum*, does not tolerate freezing but supercools substantially, despite overwintering under thick litter layers. Its mean supercooling points (SCPs) in winter are -18.2°C for juveniles, -13.5°C for males, -10.0°C for females, and the mean values drop to -16.9°C in both sexes after starvation (DAVID & VANNIER, 1996). However, the species has a wide distribution in Europe, even in cold areas.

In this paper, we present very different results obtained with another species occurring specifically in the so-called Atlantic zone, the flat-backed millipede *Polydesmus angustus* Latzel, which often overwinters in logs (COURET, 1985; GEOFFROY, 1985). Freezing-tolerance was tested and SCPs were measured for all the post-embryonic stadia, and winter survival was compared in cultures under two

thermal regimes, with or without moderate frost. It appears that *P. angustus* is not freezing-tolerant while showing a poor supercooling ability – in other words it has virtually no physiological protection against freezing. This strategy is consistent with microclimatic data from an overwintering site and may be advantageous, although costs in terms of winter survival cannot be ruled out.

Our findings, together with data on spiders (KIRCHNER, 1987), woodlice (TANAKA & UDAGAWA, 1993) and soil millipedes (DAVID & VANNIER, unpublished), suggest that the adaptive patterns assumed by DANKS (1978) may be more readily perceived in classes of arthropods other than insects.

MATERIALS AND METHODS

The millipede species

The millipedes used in the present study were derived from the laboratory grounds, a mixed deciduous wood located at Brunoy (France), about 20 km southeast of Paris. *P. angustus* (Polydesmida: Polydesmidae) is the only representative of the genus *Polydesmus* on the site. The species passes through eight post-embryonic stages (stadia), the last of which is the adult. Individuals can be assigned to a stadium by counting the number of body rings, and sexed from stadium IV onwards depending on whether the 8-th pair of legs is present (females) or absent (males). Breeding occurs in spring and summer and the life cycle lasts one or two years according to individuals (DAVID *et al.*, 1993). In short, individuals with a 1-yr cycle overwinter once, mainly as stadium VII, and those with a 2-yr cycle overwinter twice, the first time mainly as stadium V and the second as adults. Pitfall trapping has shown that activity is reduced but not stopped in winter, while stadial growth is completely arrested (DAVID *et al.*, 1993).

Determination of SCP and tolerance to freezing

The supercooling point (SCP), *i.e.* the temperature at which body water spontaneously freezes when a specimen is cooled, was measured for 137 specimens of both sexes, belonging to all stadia. 93 individuals were taken from a stock culture kept in a controlled-temperature cabinet under the seasonal conditions described below (table 1-b). The culture was started in July 1993 with stadium I individuals produced by three adult females from the field. SCPs were measured in stadia I to V during the summer, then in stadia V to VII in December.

17 individuals of stadia VI and VII were collected directly from the field during the winter of 1994-95, to compare their SCPs with those of laboratory-reared individuals overwintering in the same stadia. The SCPs of adults were measured for 11 field specimens collected during the same period.

The effect of starvation on SCP was studied using 16 individuals of stadium V taken from the stock culture in December and kept on moist filter paper at 4°C in the dark, for three weeks, before being processed.

SCP was determined by putting millipedes individually in a steel chamber (3.0 cm in diameter) which was cooled by a cryostat at the standard rate of 1°C min⁻¹. The specimens were wrapped in aluminium foil in close contact with a nickel-chromium thermocouple (0.25 mm in diameter), recording the exotherm produced at SCP by conductivity. The output of the thermoelectrical unit (sensitivity 0.1°C) was continuously displayed on a potentiometric chart recorder and cooling was stopped following the rebound of the curve, at or a few degrees below the SCP temperature (VANNIER, 1994).

After freezing exposure the millipedes were weighed to the nearest 10 µg (fresh weight) and allowed to recover on moist filter paper for various periods (48 h at the maximum), either at 4°C or at room temperature. Specimens which did not resume moving were considered to be dead. Stadium and sex were then determined under a stereomicroscope.

TABLE I. – Monthly maximal and minimal temperatures in two separate cultures of *Polydesmus angustus*: (a) in an unheated room in 1992-93 and (b) in a controlled-temperature cabinet.

Month	(a) Unheated room		(b) Cabinet	
	Max. T (°C) recorded	Min. T (°C) recorded	Day T (°C) (8 a.m./8 p.m.)	Night T (°C) (8 p.m./8 a.m.)
June	27	9	18	14
July	28	16	20	16
August	27	12	20	16
September	23	8	18	14
October	16	3	14	10
November	17	1	9	5
December	10	–6	5	3
January	14	–3	4	2
February	–	–	5	3
March	–	–	9	5

Susceptibility to chilling

In order to determine whether millipedes could be killed or fatally injured at temperatures above their SCP (BALE, 1987), a further sample of 20 adults and subadults of both sexes (3 of stadium VIII, 17 of stadium VII) was collected from the field in late November 1995. Individuals were cooled at the rate of $1^{\circ}\text{C min}^{-1}$ under the same conditions as for SCP determination, but cooling was stopped before the millipedes were frozen, at temperatures ranging from -2 to -6°C . In most cases the SCP was not reached so that the animals were briefly exposed to chilling only. In five out of 20 cases the SCP was reached, but cooling was not continued down to the SCP temperature following the exotherm, so that freezing was only initiated (BLOCK, 1991). Each specimen was then kept at 5°C on moist filter paper for at least 72 h, and inspected daily. Dead leaves were added to enable the millipedes to feed.

After three to five days the surviving specimens were tested for freezing-tolerance as above, by further cooling down to temperatures slightly below the SCPs.

Survival in culture

Millipedes were reared in transparent plastic boxes with tight lids on a layer (about 1 cm deep) of moist sieved soil. They were fed on leaf litter rinsed in distilled water, regularly replaced and either supplemented or not with a pinch of dry food yeast (*Saccharomyces cerevisiae*)¹. Two treatments were applied.

Treatment a: Stadium I individuals, hatched from five different nests from May to August 1992, were put in boxes supplemented with yeast. The nests had been built in the laboratory by adult females from the field. The boxes were kept in an open, unheated room in which the photoperiod conditions were normal and temperatures approximately followed those outdoors (table I-a). The temperatures were recorded weekly using a maximum and minimum thermometer (accuracy $\pm 1^{\circ}\text{C}$). In October 1992, 102 individuals of stadia IV to VII were divided into seventeen 200 ml boxes. In November and December the boxes were inspected; animals active near the surface were counted and their stadium determined. In January 1993, after the first period of frost, the boxes were emptied to estimate survival.

Treatment b: Stadium I individuals hatched from four different nests from May to July 1993 were put in boxes, half of which were supplemented with yeast. The nests had been built in the laboratory

¹ The addition of yeast formed part of another study.

by adult females from the field. The boxes were kept in a controlled-temperature cabinet fitted with a glass door to maintain normal photoperiod conditions. Temperature was adjusted to the monthly mean temperature of the region, with a daily range limited to 4°C (mean $\pm 2^\circ\text{C}$) from spring to autumn and 2°C (mean $\pm 1^\circ\text{C}$) in winter (table I-b). Millipedes thus overwintered without undergoing sub-zero temperatures. In October 1993, 184 individuals of stadia V to VII were divided into sixteen 400 ml boxes without yeast, and 176 individuals of stadia VI to VII (adults) into sixteen 400 ml boxes with yeast. In March 1994, the boxes were emptied to estimate winter survival.

Microhabitat temperatures

Winter temperatures of a rotting log inhabited by *P. angustatus* were taken at Brunoy between 13th January and 12th March 1995. The log was about 40 cm in diameter. Temperatures were measured (i) in the air just above the log; (ii) deep in the log, by forcing a probe into gaps in the rotting wood; and (iii) in the soil just beneath the log. The probes were connected to a 3-channel chart recorder (accuracy $\pm 0.5^\circ\text{C}$).

RESULTS

Tests of freezing tolerance and SCP temperatures

Under the experimental conditions used, no specimen survived freezing exposure, even when care was taken to stop cooling at the SCP temperature following the exotherm. Small movements of appendages were sometimes observed during thawing but no individual was able to resume activity, even after 48 h. Typically, millipedes remained motionless, with the rectum everted.

SCP values (mean, standard error and range) are given for each of the eight stadia in table II. The two sexes are pooled from stadium IV onwards, because there was no significant difference between the SCPs of males and females, whether tested for each stadium or for the whole sample. Overall, the mean SCPs of fed specimens were $-5.5 \pm 0.2^\circ\text{C}$ for males ($n = 44$) and $-5.2 \pm 0.2^\circ\text{C}$ for females ($n = 39$).

TABLE II. – Supercooling data (mean, standard error and range) for each stadium of *Polydesmus angustatus* under various conditions. Significance of differences between samples is discussed in the text (n : sample size).

Stadium	Origin	Season	Supercooling point ($^\circ\text{C}$)		
			n	Mean $\pm$ SE	Range
I	Culture	Summer	12	-7.4 ± 0.5	-10.6 to -5.2
II	Culture	Summer	14	-6.9 ± 0.4	-9.7 to -4.9
III	Culture	Summer	12	-6.7 ± 0.1	-7.5 to -5.8
IV	Culture	Summer	12	-6.9 ± 0.4	-9.7 to -5.6
V	Culture	Summer	10	-5.7 ± 0.2	-6.5 to -4.8
"	Culture	Winter	11	-5.4 ± 0.2	-6.3 to -4.3
"	Culture/Starved	Winter	16	-4.9 ± 0.1	-5.6 to -4.3
VI	Culture	Winter	11	-5.3 ± 0.1	-5.6 to -4.8
"	Field	Winter	5	-6.1 ± 0.7	-7.5 to -3.5
VII	Culture	Winter	11	-5.7 ± 0.2	-7.2 to -4.9
"	Field	Winter	12	-4.6 ± 0.4	-6.5 to -2.2
Ad	Field	Winter	11	-3.5 ± 0.3	-5.5 to -2.2

(Student's t -test: $t = 0.75$, $P = 0.46$). In the winter samples, the mean SCP was $-5.2 \pm 0.2^\circ\text{C}$ for both males ($n = 30$) and females ($n = 31$) ($t = 0.10$, $P = 0.92$).

A comparison between individuals of stadia VI and VII overwintering in culture and in the field gave no evidence of a change in SCP due to laboratory conditions. The difference between the two sample means ($-5.5 \pm 0.1^\circ\text{C}$ for $n = 22$ in culture vs. $-5.1 \pm 0.4^\circ\text{C}$ for $n = 17$ in the field) was not significant ($t = 1.34$, $P = 0.19$).

SCP tends to increase with larger body size. In the whole group of fed individuals, the slope of the linear regression between SCP and ring number (RN) was significantly greater than zero ($\text{SCP} = -8.36 + 0.20 \text{ RN}$; $n = 121$, $R^2 = 0.41$, $P < 0.001$) (fig. 1); likewise, the slope of the linear regression between SCP and fresh weight (FW) was significantly greater than zero ($\text{SCP} = -6.39 + 0.06 \text{ FW}$; $n = 121$, $R^2 = 0.37$, $P < 0.001$). Although the SCPs of the smallest individuals were measured in summer and those of the largest individuals in winter (table II), the variations appear to result more from body size than from season. Evidence of this is given by the fact that there was no significant difference between the SCPs of stadium V individuals in summer ($-5.7 \pm 0.2^\circ\text{C}$, $n = 10$) and winter ($-5.4 \pm 0.2^\circ\text{C}$, $n = 11$) ($t = 1.34$, $P = 0.19$). In addition, the within-season regressions of SCP on body size (ring number) were significant, both for stadia I to V in summer ($\text{SCP} = -7.85 + 0.13 \text{ RN}$; $n = 60$, $R^2 = 0.13$, $P < 0.01$) and for stadia V to VIII in winter ($\text{SCP} = -10.91 + 0.36 \text{ RN}$; $n = 61$, $R^2 = 0.16$, $P < 0.01$).

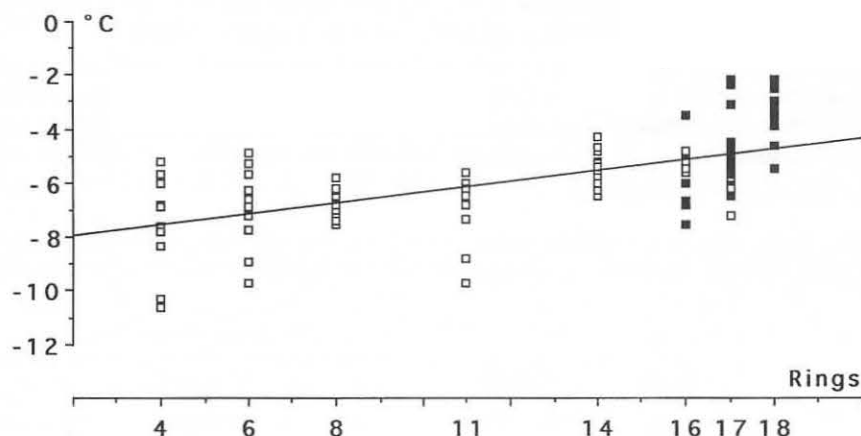


FIG. 1. – Linear regression of the supercooling point ($^\circ\text{C}$) on body size (number of podous rings) for fed specimens of *Polydesmus angustus* belonging to the eight stadia. Values from a laboratory rearing ($\square$) and from the field ($\blacksquare$) are shown separately but were pooled for calculating the regression equation: $y = -8.36 + 0.20x$.

The specimens of stadium V starved for three weeks in winter showed no depression of SCP. On the contrary, their mean SCP was significantly higher than that of stadium V individuals overwintering in the presence of food ($-4.9 \pm 0.1^\circ\text{C}$ for $n = 16$ vs. $-5.4 \pm 0.2^\circ\text{C}$ for $n = 11$, respectively) ($t = 2.73$, $P < 0.05$).

Tests of susceptibility to chilling

No pre-freezing mortality occurred during cooling at $1^{\circ}\text{C min}^{-1}$. The 20 specimens tested (three at -2°C , four at -3°C , six at -4°C , five at -5°C and two at -6°C) survived for at least 72 h after the test and remained active; several resumed feeding. Even those which reached the SCP and were removed from the exotherm peak (five out of 20) survived and remained active.

The check carried out after a few days showed that none of these 20 specimens were freezing-tolerant, since all were killed after freezing slightly below the SCP temperature.

Winter survival in culture

The percentage survival values were dramatically different between the two treatments.

Treatment a: In the unheated room, brief inspections of boxes showed that the overall rate of survival since October was $\geq 71\%$ in November and $\geq 63\%$ in December. The sample included individuals of stadium VII (54% of observations in November and 56% in December), stadium VI (28% of observations in November and 22% in December) and a few of stadia V and VIII. After two successive weeks in which temperature decreased to c. -6°C (28 Dec. to 4 Jan.) and -3°C (4 Jan. to 11 Jan.), all individuals were dead in January.

Treatment b: In controlled-temperature cabinet without frost, the overall rate of survival from October to March was 92% in the boxes without yeast and 96% in the boxes supplemented with yeast.

These data strongly suggest that the moderate frosts resulted in the death of all millipedes in treatment a.

Microhabitat temperatures

The results are given in table III. The 1994-95 winter was very mild and, for two months, only 20 frost days were recorded at ground level. On seven occasions the temperature decreased between -4 and -5°C —which theoretically threatened a number of *P. angustus* individuals, 36% of the winter SCPs being higher than or equal to -5°C . However, there was no frost within the log, the coldest temperature recorded being 1°C . Thermal buffering was even more considerable in the soil below the log, where the coldest temperature recorded was 4°C .

TABLE III. — Thermal buffering within and below a decaying log inhabited by *Polydesmus angustus*, during two winter months at Brunoy, France (January-March 1995).

Probe location	Number of frost days	Cumulative frequency distribution of days of frost below:				
		-4°C	-3°C	-2°C	-1°C	0°C
In the air above the log	20	7	8	11	15	20
Within and below the log	0	—	—	—	—	—

DISCUSSION

Freezing status and level of cold-hardiness

The supercooling profile of *P. angustus* resembles that of many freezing-tolerant species: (i) SCPs are very high with individual figures up to -2.2°C ; (ii) there is no seasonal variation in supercooling ability, the most abundant stadia in winter – stadia V to VIII (DAVID *et al.*, 1993) – in fact showing higher SCPs than juveniles in summer, probably because of their larger body size; (iii) potent ice-nucleating agents may be present in the haemolymph in so far as SCP was not depressed after three weeks of starvation in the laboratory. However, *P. angustus* is apparently not tolerant of freezing, since no specimen survived cooling below the SCP.

The absence of freezing-tolerance in *P. angustus* is not an artefact – due for instance to the rates of cooling and rewarming in the laboratory (BAUST & ROJAS, 1985; BLOCK, 1991). We not only tested some specimens using cooling rates slower than $1^{\circ}\text{C min}^{-1}$ (0.2 and $0.1^{\circ}\text{C min}^{-1}$), without obtaining post-freezing survival, but also found that all stadia from fifth to adult died after cooling to *c.* -6°C in an unheated room (treatment a), experiencing rates of cooling and rewarming close to those in the field. Therefore, even if particular conditions enabling individuals to survive freezing were to be found, freezing-tolerance would by no means be a common phenomenon in this species. Limited freezing-tolerance has been reported in centipedes (Chilopoda) (CRAWFORD & RIDDLE, 1974; TURSMAN *et al.*, 1994), requiring inoculative freezing by external ice in *Lithobius forficatus*. HAACKER (1968) tested the survival of *P. angustus* under conditions likely to result in inoculative freezing, but no specimen survived a brief exposure to -6°C . We thus conclude that the flat-backed millipede has virtually no physiological protection against freezing.

Although the species easily survives low temperatures above 0°C , individuals begin to die as soon as the temperature decreases by a few degrees below 0°C . During the supercooling experiments at the rate of $1^{\circ}\text{C min}^{-1}$, it is clear that death resulted from freezing, not from chilling, and that *P. angustus* cannot be described as chilling-susceptible (*sensu* BALE, 1993). The level of chilling-tolerance remains to be studied more precisely, by assessing the survival of millipedes during longer exposures to sub-zero temperatures above the SCP. In the culture subjected to frosts around -6°C (treatment a), it is possible that a proportion of the population died without freezing; indeed, there were no survivors, whereas 9% of the winter SCPs were below -6°C in the stock culture. On the other hand, the cooling rates in an unheated room may have resulted in slightly higher SCPs than in the laboratory, for the temperature of freezing gradually rises as the rate of cooling decreases (SALT, 1966). Depending on the level of chilling-tolerance, *P. angustus* could be classified as either freezing-intolerant in the strict sense (if mortality occurred only below the SCP), or more or less chilling-tolerant (if a proportion of the population was killed before freezing in long-term exposures to cold) (BALE, 1993). In either case, however, the main feature of the species is the possibility of lethal freezing at relatively high sub-zero temperatures (on average -5°C when cooled at the standard rate), resulting in a low level of cold-hardiness.

Benefits and costs of the overwintering strategy

The lack of physiological protection against freezing may be advantageous, because even the most widespread process of resistance – avoidance of freezing by supercooling – implies costs. Irrespective of the accumulation of biological antifreezes, ice-nucleating agents present in the gut or in other body compartments have to be removed or inactivated to avoid freezing. Many arthropod species cease feeding in early winter, thereby greatly enhancing their ability to supercool (SALT, 1961; SØMME, 1982; CANNON & BLOCK, 1988), but the cost of fasting is obvious, especially in terms of lipids. In the millipede *P. germanicum*, individuals forced to starve for two months showed significant decreases in both SCP and percentage fat content (DAVID & VANNIER, 1996). In contrast, species which do not rely on cold-hardiness to survive winter can continue to exploit available food and not exhaust their lipid reserves. *P. angustus* occasionally feeds at 3°C in culture – as shown by the production of faeces – and it is possible that individuals feed throughout winter in the field, where temperatures generally rise well above zero in the afternoon. It should be noted, however, that the advantage conferred on the species by winter feeding may not hold for millipedes such as julids, which burrow deep into the soil, far from food resources.

Is the lack of cold-hardiness costly in terms of survival? *P. angustus* shows an essentially Atlantic distribution in western Europe, being among the most common Diplopod species in the British Isles, France and Belgium (KIME, 1990). Overall, this pattern accords with its response to cold, since the eastern limit of the Atlantic zone does not overlap the 0°C January isotherm (LEMEE, 1967). However, even near the centre of the species' range, it is not uncommon for the air temperature to drop below -5°C in winter and frosts are even more severe at ground level (GEIGER, 1959). The millipede must rely on its behaviour to find microsites protected from sub-zero temperatures. In the Atlantic zone, frost is not sustained enough to penetrate deep into the forest floor, which offers protected microhabitats, but *P. angustus* does not favour sites with a thick litter layer (DAVID, 1990) and the lateral projection of its legs restricts burrowing in compact soil (MANTON, 1953). Flat-backed millipedes are rather well suited to push into crevices and, in fact, *P. angustus* is commonly found under the bark or within the decaying wood of logs and stumps (COURET, 1985; GEOFFROY, 1985).

There have been conflicting opinions as to whether this type of microhabitat is as thermally buffered as the soil. According to SAVELY (1939) the protection afforded by bark to hibernating insects is very slight, but BAUST (1976) has shown that a rapid decrease in ambient temperature is substantially delayed at a depth of only a few centimeters in decayed wood. Therefore, during a short spell of frost, the temperature within a log may not fall below 0°C, as confirmed by our field measurements. Logs offer a suitable microhabitat for *P. angustus* overwintering in the Atlantic zone—at least for surviving unfavourable temperature conditions, irrespective of problems raised by the occurrence of competitors and predators in the same microsites.

Nevertheless, the possibility that frost reaches individuals living under thin litter layers and bark cannot be completely excluded, even near the centre of the species range. This can occur during unusually cold spells, when temperatures in the air, the upper soil layers and logs tend to equilibrate below 0°C. There may at times be a high mortality in winter populations of *P. angustus*, which might

explain the substantial fluctuations in population density observed in this species (BLOWER, 1970; DAVID, 1990).

Towards the edges of the species' range – in Denmark and western Norway (MEIDELL & ENGHOF, 1993), or at middle altitudes in Switzerland and western Austria (PEDROLI-CHRISTEN, 1993; TADLER & THALER, 1993) – winter conditions are harsher and it is not clear how *P. angustus* manages to survive. It becomes increasingly difficult to find surface microhabitats protected from frost, especially in the absence of an insulating snow cover (GEIGER, 1959). It is not known whether peripheral populations show different physiological adaptations, such as lower SCPs than those we have measured; or whether they rely on man-made sites for surviving; or, finally, whether they frequently become extinct, the species being reintroduced and spreading sporadically in favourable years.

Adaptive patterns in relation to microhabitats

The absence of resistance to freezing found in *P. angustus* supports DANKS' (1978) hypothesis, but why should this feature not be more common in arthropods protected from frost by their behaviour? A first aspect already noted by DANKS is that, in general, adaptive patterns are too complex to be explained on the basis of a single selection pressure. Plausible, adaptive arguments for the persistence of cold-hardiness in sheltered microsites can be put forward. For example, in species which overwinter in protected but not completely predictable microhabitats – such as logs in the temperate zone – occasional costs in terms of survival may balance the benefits of being winter active without cold-hardiness; hence, selection may favour a cold-hardy stage in certain log-dwellers and not in others. One can also speculate that, in species which overwinter in protected and predictable microhabitats – e.g. deep layers of well-drained soil – resistance to freezing is a fixed character and burrowing behaviour is actually related to a factor other than cold, such as predation. Thus, the persistence of a high degree of cold-hardiness in thermally-buffered environments cannot be excluded *a priori*.

Furthermore, a particular option may be favoured in certain taxa and not in others due to phylogenetic constraints. In the case of temperate insects overwintering in the soil the evidence is conflicting, but many species supercool regardless of need (SØMME, 1982). This originates notably from (i) the existence of a stage with an inherently low SCP, the pupa of holometabolous insects, and (ii) the phenomenon of winter diapause, widespread in temperate insects, which often results in an increased cold-hardiness (DENLINGER, 1991).

However, adaptive patterns more consistent with DANKS' (1978) hypothesis may emerge from studies on non-insectan arthropods. Several species of spiders (Araneida) living under rocks in Germany exhibit the same features as *P. angustus*, with no freezing-tolerance and mean SCPs between -4 and -7°C , in contrast with species found in more exposed habitats (KIRCHNER, 1987). As for the soil macrofauna, the woodlouse *Porcellio scaber* (Isopoda) overwinters with a low level of cold-hardiness (TANAKA & UDAGAWA, 1993), and preliminary investigations of four species of julid and glomerid millipedes from forest soils in eastern France have led to similar results (DAVID & VANNIER, unpublished). A comparative approach might be fruitful when more data are available for these somewhat neglected taxa.

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